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In vivo Soft Tissue Differentiation by Diffuse Reflectance Spectroscopy: Preliminary Results

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Abstract

Remote laser surgery does not provide haptic feedback to operate layer by layer and preserve vulnerable anatomical structures like nerve tissue or blood vessels. The aim of this study is identification of soft tissue *in vivo* by diffuse reflectance spectroscopy to set the base for a feedback control system to enhance nerve preservation in oral and maxillofacial laser surgery. Various soft tissues can be identified by diffuse reflectance spectroscopy *in vivo*. The results may set the base for a feedback system to prevent nerve damage during oral and maxillofacial laser surgery.

Keywords; Feedback system; soft tissue; tissue differentiation; diffuse reflectance; linear discriminant analysis; *in vivo*.

1. Introduction

Laser surgery has a number of advantages [1]. An ability to work remotely provides high precision, little trauma and high level sterility can be guaranteed. However, remote laser surgery does not provide haptic feedback to operate layer by layer and preserve vulnerable anatomical structures like nerve tissue or blood vessels [2]. Thus, additional means are required to guide the laser surgeon to reduce side effects and complications. Several approaches for tissue specific laser ablation control by optical feedback systems have been described [3-5].

Diffuse Reflectance Spectroscopy (DRS) provides a straightforward and simple approach for optical tissue differentiation. Differentiation between healthy soft tissues *ex vivo* has been successfully done by analyzing diffuse reflectance spectra [6]. The goal of this study is to differentiate several types of healthy soft tissue by diffuse reflectance spectroscopy with special emphasis on identification of nerve tissue from soft tissues, while the preservation of nerve tissue is crucial for oral and maxillofacial laser surgery.

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2. Materials and methods

1.1. Animal preparation

Charles River rats were used for the *in vivo* experiment. Intraperitoneal anesthesia with 75 mg/kg Ketaminhydrochlorid (Ketavet®) and 10 mg/kg Xylazin (Rompun®) were applied to the rats and individually adapted to the body weight. After complete anesthesia, the right lower limb was shaved and disinfected. The following operative techniques were performed under strictly aseptic conditions. The tissue of interest was prepared by scalpels. Haemostasis was achieved by electrocauterization of the blood vessels. After performing the optical measurements we sacrificed the animals under running anaesthesia by an intraperitoneal injection of Brevimythal®.

1.2. Experimental setup

Diffuse reflectance spectra of nerve tissue, skin, muscle and fat tissue were acquired from Charles River rats *in vivo*. Influence of non-healthy tissue was not considered in this study. For every single tissue specimen, 150 diffuse reflectance spectra were acquired (5 various spots times 30 spectra per spot). The experimental setup consisted of a pulsed Xenon lamp PX-2® (Ocean Optics, USA) projected onto the tissue via the reflection-backscattering probe, and a scientific grade spectrometer QE65000® (Ocean Optics, USA) with 2.3 nm optical resolution which was used for the detection. The spectrometer has 30 dB S/N and 44 dB dynamic range. The Reflection-backscattering probe QR600-7-SR-125F® (Ocean Optics, USA) consists of six illumination fibers and a single collection fiber was used. Each optical fiber has 600 μm core diameter and 0.22 numerical aperture. The raw spectra were processed to calculate diffuse reflectance spectra corrected by stray light and light source emission spectra.

Tissue differentiation was performed by the linear discriminant analysis (LDA). Specificity and sensitivity were calculated by the receiver operating characteristic (ROC) analysis and using the area under curve (AUC) parameter. The area under the ROC curve (AUC) provides a quantitative judgment of the ultimate differentiation accuracy [7]. The more accurate the differentiation between the two tissue types, the closer the corresponding AUC approximates "1".

3. Results

Figure 1 shows typical diffuse reflectance spectra collected from four various soft tissue types. The spectral signatures from the tissues have not been found obviously distinctive and demonstrated rather subtle peculiarities. The wiggles on the spectra are due to the noise from the spectrometer, because the spectra did not experience any noise reduction processes. Standard deviation and averaging process were not needed because every single spectrum was considered for differentiation.

By using LDA, we found that the optimal differentiation between nerve and the rest of the soft tissues can be provided within the 350-420 nm range. The differentiation between the rests of soft tissues can be facilitated by exploiting the 350-400 nm range. The broader range of wavelengths for differentiation does not mean that the differentiation between tissues is worst. This range is only covering the range where the chosen wavelengths for differentiation are located and it does not mean that the whole wavelengths are needed for differentiation. For example with nerve and fat, only 8 wavelengths within the 350-420 nm range were needed to provide optimal differentiation.

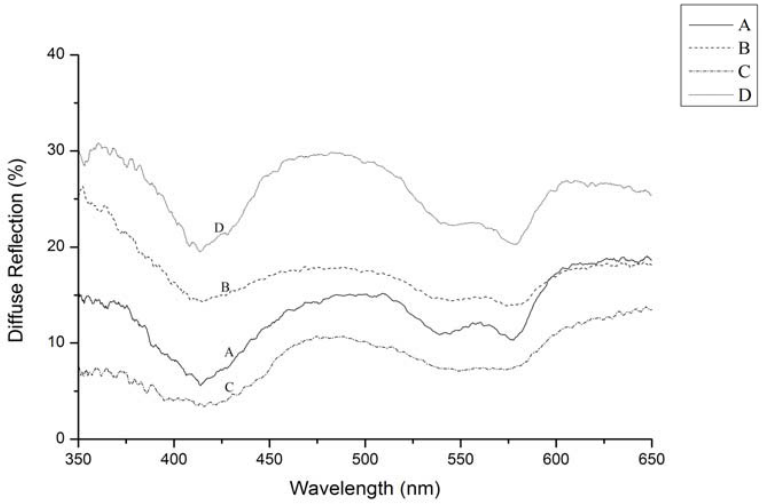

Fig. 1. Typical diffuse reflectance spectra from fat (A), muscle (B), nerve (C) and skin (D).

We calculated the sensitivity and specificity using the ROC analysis [7]. The sensitivity and specificity values used for the discrimination of the four tissues studied are given in Table 1 and 2. The AUC also was computed to judge the accuracy of the discrimination method selected. The AUC values used for the discrimination of the four tissues studied are given in Table 3.

Table. 1. The sensitivity of discrimination between four tissue types.

Sensitivity	Skin	Muscle	Fat	Nerve
Skin				
Muscle	1.000			
Fat	0.989	0.947		
Nerve	1.000	0.992	0.844	

Table. 2. The specificity of discrimination between four tissue types.

Specificity	Skin	Muscle	Fat	Nerve
Skin				
Muscle	1.000			
Fat	0.997	0.874		
Nerve	0.992	1.000	0.997	

Table. 3. Area under the ROC curve (AUC). Discrimination between four tissue types.

AUC	Skin	Muscle	Fat	Nerve
Skin				
Muscle	1.000			
Fat	1.000	0.920		
Nerve	1.000	1.000	0.983	

4. Conclusions

Nerve tissue could correctly be identified and differed from the skin, muscle and fat tissues at more than 90% of the cases (AUC results) with a specificity of over 85% and a sensitivity of more than 80%. Our results show that various soft tissues can be identified by diffuse reflectance spectroscopy *in vivo*. The results may set the base for a feedback system to prevent nerve damage during oral and maxillofacial laser surgery. However, further studies and more data set have to be collected to validate the reproducibility and discrimination accuracy.

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